(0–1 cm) depth and when water leaching from paddy soil was high (3–4 cm).

The downward movement of K-11451 in paddy soil was investigated by adding the compound at rates varying from 1.56 to 50 g ha^{-1} to the top of a column of paddy soil (10 cm) from which water subsequently leached at 3 cm per day. After 24 h leaching, the soil column was divided into 1-cm sections, starting at the top, and barnyardgrass seeds were planted in each section. Measurements of the extent of growth of the weed indicated that the compound had moved readily to a depth of 3 cm. The half-life of K-11451, estimated in a pot test using Gerber's method,[5] was relatively short, being 15.2 days under submerged paddy conditions.

It has been reported that phytotoxic effects of several sulfonylurea compounds on rice can be reduced by using slow-release formulations and by using them as a mixture with daimuron.[6] The safety of treated direct-seeded or transplanted rice was increased when K-11451 was applied at 9 or 18 g ha^{-1} either once as a slow-release formulation or with daily applications of 5.0, 4.0 or 3.3% of the total amount. It was also increased when K-11451 was applied in admixture with daimuron; for example, K-11451 + daimuron at 9 + 250 g AI ha^{-1}, gave complete control of annual weeds, except *Aneilema keisak* Hassk, in a greenhouse trial, without injury to the rice (Table 1). It is well known that *A keisak* cannot be controlled adequately with sulfonylurea herbicides, including K-11451, so that control of this weed without damage to rice would require mixtures with other herbicides. Of a number of mixtures, the combination of K-11451 with mefenacet and daimuron (9 + 250 + 250 g AI ha^{-1}) provided the best results. Thus such a combination could be used successfully as a one-shot herbicide in rice culture.

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Chloroacetanilides, oxyacetamides, tetrazolinones: mode of action. 1. Cross resistance and oleic acid incorporation in algal model systems

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Abstract: Results presented suggest that chloroacetamides, oxyacetamides, tetrazolinones, and possibly cafenstrole, act at the same site, although the precise molecular mode of action is still unknown, despite much research effort.

Keywords: herbicides; chloroacetanilides; oxyacetamides; tetrazolinones; oleic acid incorporation; mefenacet; BAY FOE 5043; BAY YRC 2388; cafenstrole

1 INTRODUCTION

In 1984, oxyacetamide herbicide chemistry was first introduced with the herbicide mefenacet, used mainly for the control of *Echinochloa* species in rice.[1] In 1995, the oxyacetamide BAY FOE 5043 was added as a second herbicide from this group, this time for weed control in corn, soybean and cereals.[2] The first commercial tetrazolinone herbicide will be BAY YRC 2388, which was introduced for applications similar to those of mefenacet in 1997.[3]

Extensive studies on the mode of action of mefenacet have shown close similarities with chloroacetanilide herbicides, e g alachlor. The results and effects obtained with mefenacet have now been reproduced with BAY FOE 5043, BAY YRC 2388, and also with cafenstrole. These are:

1. Inhibition of mitotic entry in oat roots.[4]
2. Inhibition of cell division in the green alga *Chlamydomonas reinhardtii* Dang.[5]
3. Symptomology in corn: strong plant stunting and leaf curling.
4. Complete reversal of the inhibitory effects in corn by simultaneous treatment with dichloroacetamide safeners.
5. Metabolism in corn by glutathione conjugation, particularly in safened plants, suggests facile nucleophilic displacement (not studied for cafenstrole).
6. Plant sensitivity microtests aimed at defining the relevant mode of action group place the oxyacetamides, tetrazolinones and cafenstrole with the chloroacetamides.[6]

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Table 1. Inhibition of organogenesis and growth in several microtests aimed at finding the relevant mode of action group

Herbicide	pI_{50}[a] SRL	SRD	ORL	OLL	ORD
Alachlor	5.0	<4	5.6	4.3	5.6
BAY FOE 5043	4.9	<4	8.4	5.7	7.9
Mefenacet	4.7	<4	6.5	5.4	7.0
BAY YRC 2388	5.3	4.6	6.8	5.3	5.8
Cafenstrole	7.4	5.4	7.1	6.2	6.5

[a] Based on % inhibition data.
SRL and SRD: root regeneration in light and darkness in soybean seedling cuttings; ORL, OLL and ORD: root regeneration and leaf growth, respectively, in light and darkness in oat seedling cuttings.[6]

Table 2. Sensitivities of *Chlamydomonas reinhardtii* wild-type (wt) and a resistant strain (tfl3+) selected against BAY FOE 5043 towards a selection of herbicides with different modes of action[a]

Herbicide	pI_{50} wt	pI_{50} tfl3+	Resistance factor
Group I:			
BAY FOE 5043	6.1	4.3	63
BAY YRC 2388	5.2	3.2	100
Cafenstrole	5.5	<3.2	>200
Alachlor	6.0	4.2	63
Mefenacet	5.1	3.6	32
Butachlor	7.3	5.5	63
Group 2:			
Amiprofos-methyl	6.6	5.9	5
Atrazine	5.4	5.2	1.6
Bensulfuron	5.9	5.7	1.6
Chlorpropham	5.7	5.2	3.2
Diflufenican	8.8	8.1	5
Diuron	6.4	5.5	8
Fluorodifen	6.8	6.3	3.2
Flurtamone	5.7	5.5	1.6
Methabenzthiazuron	5.7	5.5	1.6
Metribuzin	6.4	6.5	0.8
Paraquat	6.4	6.3	1.3
Pentachlorophenol	4.8	4.6	1.6
Sulfometuron-methyl	6.6	6.4	1.6

[a] In general only low resistance factors are found. It is suggested that permeation changes are the reason for a low level of unspecific resistance.[7]

7. *Chlamydomonas reinhardtii* strains selected for resistance to mefenacet, metazachlor or metolachlor were cross-resistant to all other oxyacetamides and chloroacetanilides, but not to herbicides with other (known) modes of action.[7] A new strain tfl3+ selected for resistance to BAY FOE 5043 after mutagenesis with MNNG was similarly cross-resistant to all oxyacetamides and chloroacetamides, and also to tetrazolinones (BAY YRC 2388) and cafenstrole.
8. Incorporation of [1-^{14}C-]oleic acid into sporopollenin in the green alga *Scenedesmus acutus* was specifically inhibited by oxyacetamides, tetrazolinones, chloroacetamides, and cafenstrole.[8]
9. The growth inhibition caused in parsley cell suspension cultures by oxyacetamide, tetrazolinone or chloroacetamide herbicides can be completely reversed with a combination of the amino acids proline and arginine.[9]

2 METHODS

The microtest methods, including *Chlamydomonas* growth measurements[6] and the oleic acid studies with *Scenedesmus*,[8] have been described previously. Mutagenisis of *Chlamydomonas* cells with MNNG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) was done according to Harris.[10] Cells were subsequently selected for resistance in 10 mg litre^{-1} BAY FOE 5043 on agarose. The resistance obtained is stable over several years in cells grown in the absence of the herbicide.

3 RESULTS AND DISCUSSION

The molecular mode of action of the herbicides studied here is not known, despite much research effort over the past decades. Table 1 shows inhibition in several plant growth and regeneration tests by the compounds studied here. A particular sensitivity is seen in the oat (*Avena sativa* L) seedling cuttings. All compounds are more inhibitory than phytotoxic and show a similar spectrum of activity. In Table 2 the sensitivities of wild-type and resistant *C. reinhardtii* cells are compared. Only herbicides of the title group are cross-resistant. In Table 3 a high sensitivity of the [^{14}C]oleic acid incorporation test is documented. This test has been shown to be highly specific for the action of chloroacetanilide herbicides.

Taken together, the results presented here suggest that chloroacetamides, oxyacetamides, tetrazolinones, and possibly cafenstrole, act at the same site. Detailed results for the oleic acid incorporation and amino acid

Table 3. Inhibition of [1-^{14}C]oleic acid incorporation into a non-lipid fraction of the alga *Scenedesmus acutus* after 0.5+3 h (herbicide alone followed by herbicide plus [^{14}C]oleic acid)

Herbicide	pI_{50}
Alachlor	7.0
BAY FOE 5043	6.8
Mefenacet	5.7
BAY YRC 2388	7.0
Cafenstrole	7.1

reversal tests are presented elsewhere. The resistant *C. reinhardtii* cells could present an opportunity to select the resistance gene by transformation experiments. If the resistance is due to target site alteration, which seems to be the case, this could lead to finding the target gene.

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Guttation – the basis of an assay for evaluating formulation behaviour *in vivo*

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Abstract: Guttation droplets collected from the tips of winter leaves, previously treated with a ^{14}C version of an experimental xylem-mobile fungicide (ExpF) that was known to elute readily in guttation fluid, were analysed for the presence of radiolabel. The effects of adjuvants on the elution rate was investigated and related to the known biological profile of the fungicide when used in combination with adjuvants. This method, using ExpF as a model molecule, is undergoing further development as a means of investigating formulation behaviour *in vivo*. Not all xylem-mobile fungicides elute significantly and data are presented to illustrate this.

Keywords: guttation; mobility; fungicide; adjuvant; formulation

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1 INTRODUCTION

When the dew point of air is reached, leaves can no longer lose water as vapour. To maintain transpiration under these conditions, water is lost in the liquid phase by a process called guttation. Water exudes through specialised cells called hydathodes and droplets appearing at the leaf tips or margins can contain various salts, sugars and other organic substances.[1]

In previous experiments (Harris R I, unpublished), it was noticed that an experimental xylem-mobile fungicide (ExpF) eluted in significant quantities in the guttation fluid of winter wheat seedlings. The possibility of using this molecule as a means of evaluating formulation behaviour on leaf surfaces was recognised. By incorporating ExpF in different formulation systems, and subsequently collecting and analysing guttation fluid from treated plants, it should be possible to investigate a variety of formulation properties.

This summary presents the basic methods and preliminary data using a model acetone-based formulation system. Of particular significance for further evaluation are release profiles of encapsulated formulations.

2 MATERIALS AND METHODS

2.1 Active ingredients

The fungicides used were [^{14}C] ExpF, Specific activity 6.5 MBq mg^{-1}, and [^{14}C]fluquinconazole, Specific activity 6.2 MBq mg^{-1}. Final application solutions contained the fungicide at 0.5 g litre^{-1}, composed entirely of the radiolabelled molecule. The activity of the final application solutions was approximately 3.8×10^3 Bq µl^{-1} for [^{14}C]ExpF and 3.2×10^3 Bq µl^{-1} for [^{14}C]fluquinconazole.

2.2 Adjuvants

Two adjuvants, Adj1 and Adj2, of known widely differing uptake activation properties were used. Adj1 enhances the uptake of lipophilic, high-melting-point molecules into cereal foliage, whilst Adj2 is better suited to less lipophilic molecules of lower melting points.

2.3 Plants

Winter wheat (*Triticum aestivum* L cv Avalon) seedlings, grown in John Innes compost and maintained at a nominal 20 °C and 16 h photoperiod, were used at the one to two fully expanded leaf stage.

2.4 Application and sampling

The fungicides (0.5 g litre^{-1}) in acetone + water (85 + 15 by volume), together with the appropriate